

**What is claimed is:**

1. A recombinant fungal strain, capable of expressing chitin deacetylase, comprising an expression vector that contains a suitable promoter, a nucleic acid molecule encoding chitin deacetylase, a selectable marker sequence, a secretion signal sequence, and a transcription terminator.
2. The recombinant fungal strain according to claim 1, wherein said fungal strain is a filamentous fungus.
3. The recombinant fungal strain according to claim 1, wherein said fungal strain is selected from the group consisting of the genera *Aspergillus*, *Emericella*, *Trichoderma*, *Achlya*, *Neurospora*, *Phanerochaete*, *Tolylocadium* and *Penicillium*.
4. The recombinant fungal strain according to claim 1, comprising an expression vector that contains a nucleic acid molecule encoding chitin deacetylase obtained from *Mucor rouxii*.
5. The recombinant fungal strain according to claim 1, comprising an expression vector wherein the promoter is a constitutive or an inducible promoter.
6. The recombinant fungal strain according to claim 1 comprising an expression vector, wherein the selectable marker gene is an auxotrophic marker gene or a dominant marker gene.
7. The recombinant fungal strain according to claim 1, comprising an expression vector wherein the nucleic acid molecule encoding chitin deacetylase is expressed as a translational fusion to the C-terminus of the selection marker protein such that chitin deacetylase is expressed as a C-terminal fusion protein.
8. The recombinant fungal strain according to claim 1, comprising an expression vector that further comprises additional nucleotide sequences provided at the 5' and/or 3' terminal end of the nucleic acid molecule encoding chitin deacetylase.

9. The recombinant fungal strain that has all of the identifying characteristics of deposit No IHEM 20351, deposited at the BCCM-IHEM, biomedical fungi and yeasts collection, Scientific Institute of Public Health, Louis Pasteur, Brussels Belgium on February 10, 2004.

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10. A method for producing chitin deacetylase by a recombinant fungal strain comprising the steps of:

- constructing a recombinant fungal strain capable of expressing chitin deacetylase by recombinant DNA techniques,
- preparing a culture comprising spores of said recombinant fungal strain,
- inoculating a suitable amount of spores of said recombinant fungal strain in a suitable medium and incubating said recombinant fungal strain in said medium for a suitable period of time,
- feeding for a suitable period of time said incubated recombinant fungal strain with a suitable substrate which controls proliferation of the fungal strain,
- clarifying the medium such that fungal mycelium is removed from the medium and the supernatant of the medium which comprises chitin deacetylase is retained, and
- isolating said chitin deacetylase from said supernatant by means of chromatographic techniques.

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11. The method according to claim 10, wherein a recombinant fungal strain capable of expressing chitin deacetylase is constructed which comprises an expression vector that contains a promoter, a nucleic acid molecule encoding chitin deacetylase, a selectable marker sequence, a secretion signal sequence, and a transcription terminator.

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12. The method according to claim 10, wherein said method comprises construction of a recombinant fungal strain capable of expressing chitin deacetylase, wherein said fungal strain is a filamentous fungus.

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13. The method according to claim 10, wherein said method comprises construction of a recombinant fungal strain comprising an expression vector that comprises a nucleic acid molecule encoding chitin deacetylase obtained from *Mucor rouxii*.

14. The method according to claim 10, wherein said method comprises construction of a recombinant fungal strain comprising an expression vector wherein the selectable marker gene is an auxotrophic marker gene or a dominant marker gene.

5 15. The method according to claim 10, wherein said method comprises construction of a recombinant fungal strain comprising an expression vector wherein the promoter is a constitutive or an inducible promoter.

10 16. The method according to claim 10, wherein said method comprises construction of a recombinant fungal strain comprising an expression vector wherein the nucleic acid molecule encoding chitin deacetylase is expressed as a translational fusion to the C-terminus of the selection marker protein such that chitin deacetylase is expressed as a C-terminal fusion protein.

15 17. The method according to claim 10, wherein said method comprises construction of a recombinant fungal strain comprising an expression vector that further comprises additional nucleotide sequences provided at the 5' end and/or at the 3' end of the nucleic acid molecule encoding chitin deacetylase.

20 18. The method according to claim 10, comprising the step of inoculating an amount of spores of said recombinant fungal strain comprised between  $0.5 \times 10^5$  and  $1.0 \times 10^8$  spores per ml medium.

25 19. The method according to claim 10, comprising clarifying said medium by filtration, centrifugation and/or micro-filtration such that fungal mycelium is removed from the medium.

20. A purified recombinant chitin deacetylase obtainable by a method according to claim 10.

30 21. A recombinant yeast strain, capable of expressing chitin deacetylase, comprising an expression vector that contains a nucleic acid molecule encoding chitin deacetylase, a suitable promoter and a transcription terminator.

22. The recombinant yeast strain according to claim 21, wherein said yeast strain is a methylotrophic yeast.

23. The recombinant yeast strain according to claim 21, wherein said yeast strain is selected  
5 from the genus *Pichia* or *Hansenula*.

24. The recombinant yeast strain according to claim 21, wherein said yeast strain is a *Pichia pastoris* or a *Pichia methanolica* strain.

10 25. The recombinant yeast strain according to claim 21, wherein said *Pichia pastoris* recombinant yeast strain comprises an expression vector selected from the group consisting of pFLD1 $\alpha$ , pPIC9, pHIL-S1, pPIC9K, pPIC6 $\alpha$ , pPICZ $\alpha$ -E and pPICZ $\alpha$  and wherein said *Pichia methanolica* recombinant yeast strain comprises a pMET $\alpha$  expression vector.

15 26. The recombinant yeast strain according to claim 21, comprising an expression vector that contains a nucleic acid molecule encoding chitin deacetylase obtained from *Mucor rouxii*.

27. The recombinant yeast strain according to claim 21, comprising an expression vector that contains an inducible promoter.

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28. The recombinant yeast strain according to claim 27, comprising an expression vector that contains a methanol-inducible promoter.

25 29. The recombinant yeast strain according to claim 27, comprising an expression vector that contains a *Pichia pastoris* alcohol oxidase 1 (AOX1) promoter or a *Pichia methanolica* alcohol utilization gene 1 (AUG1) promoter.

30 30. The recombinant yeast strain according to claim 21, comprising an expression vector that further comprises a selectable marker gene selected from the group consisting of *HIS4*, *ADE2*, *ADE1*, *ARG4*, *URA3* and antibiotic resistance genes.

31. The recombinant yeast strain according to claim 21, comprising an expression vector that further comprises a secretion signal sequence to direct the transport of the chitin deacetylase protein to the extracellular medium.

5 32. The recombinant yeast strain according to claim 31, wherein said secretion signal sequence is the nucleotide sequence coding for the *Saccharomyces cerevisiae*  $\alpha$ -factor prepro peptide or the nucleotide sequence coding for the *Pichia pastoris* alkaline phosphatase signal peptide or the nucleotide sequence coding for the PHA-E signal peptide from the plant lectin *Phaseolus vulgaris* agglutinin.

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33. The recombinant yeast strain according to claim 21, comprising an expression vector that further comprises additional nucleotide sequences provided at the 5' and/or 3' terminal end of the nucleic acid molecule encoding chitin deacetylase.

15 34. A recombinant yeast strain that has all of the identifying characteristics of accession number MUCL 44353, deposited at the BCCM-MUCL fungi and yeast Collection in the Scientific Institute of Public Health, Louis Pasteur, Brussels, Belgium on January 24, 2003.

20 35. A recombinant yeast multicopy strain capable of expressing chitin deacetylase, comprising more than one copy of the nucleic acid molecule encoding chitin deacetylase from *Mucor rouxii*.

36. A method for producing chitin deacetylase by a recombinant yeast strain comprising the steps of:

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- constructing a recombinant yeast strain capable of expressing chitin deacetylase according to claim 21 by recombinant DNA techniques,
  - preparing a pre-culture comprising said recombinant yeast strain,
  - inoculating a suitable amount of said pre-cultured recombinant yeast strain in a suitable fermentation medium and incubating said recombinant yeast strain in said
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- feeding for a suitable period of time said incubated recombinant yeast strain with a suitable substrate which controls proliferation of the yeast,

- supplementing for a suitable period of time the medium of the incubated recombinant yeast strain with an inducer, capable of stimulating the transcription and translation of the gene encoding chitin deacetylase,
- clarifying the medium such that yeast cells are removed from the medium and the supernatant of the medium which comprises chitin deacetylase is retained, and
- isolating said chitin deacetylase from said supernatant by chromatographic techniques.

37. The method according to claim 36, comprising inoculating between 5 and 10 % of the initial fermentation volume of said pre-cultured recombinant yeast strain in a fermentation medium.

38. The method according to claim 36, comprising supplementing said fermentation medium with a component selected from the group consisting of surfactants, metallic ions, an inducer of the expression of chitin-related enzymes and any combinations thereof.

39. The method according to claim 36, comprising feeding the incubated recombinant yeast at a rate of 5 to 10 ml of substrate per hour per liter of fermentation medium.

40. The method according to claim 36, comprising supplementing the medium of the incubated recombinant yeast with an inducer being alcohol.

41. The method according to claim 36, comprising clarifying said fermentation medium by centrifugation and micro-filtration such that yeast cells are removed from the medium.

42. A purified recombinant chitin deacetylase obtainable by a method according to claim 36.

43. The method according to claim 40, wherein the alcohol is methanol.